REMARKS

Claims 48-61, presented hereby, are pending.

Claims 1-47 have been cancelled, without prejudice or disclaimer.

Present independent claim 48 combines subject matter of claims 36 and 38. Present claim 49 contains subject matter from claim 38. Present claims 50-54 correspond to claims 39, 42-44, and 46, respectively, made dependent on present claim 48. Claim 55 is an independent claim, which represents subject matter of present claim 48 styled as a "method of treating a lesion of neuronal tissue." Present claims 56-61 correspond to present claims 49-54, but are dependent on claim 55.

Applicants wish to thank the Examiner for the indication of allowable subject matter (Office Action page 8), i.e., the suggested claim reading:

> A method of enhancing axonal regeneration comprising locally administering an inhibitor substance that inhibits basal membrane formation of lesioned postcommissueral fornix to enhance axonal regeneration, and wherein the inhibitor substance is an anti-collagen IV anitbody or α,α'-dipypridyl (DPY).

The present claims incorporate language from the aforesaid allowable claim in order to resolve issues raised in the rejection under §112, second paragraph.

Claims 36-38 and 42-47 were rejected under 35 U.S.C. §112, first paragraph, for allegedly lacking enablement and under 35 U.S.C. §112, second paragraph, allegedly being indefinite. Reconsideration is requested with respect to the rejections under §112, first paragraph, and under §112, second paragraph, in view of the changes in the claims, effected hereby, and the following remarks.

The limitation of claim 36 by incorporation of the subject matter of claim 38 is intended to resolve the rejection against the breadth of claim 36. Since, now, specific classes of compounds are recited, there is no undue burden for identifying compounds that fall within the claim scope.

The rejection against the term "improvement of the CNS" under item 9 of the Office Action (page 7) is addressed by the language in the preamble of the present claims. Of course, it was never meant to claim an improvement of the CNS after a lesion but, rather, a regeneration of neuronal tissue and, consequently, the recovery of CNS functionality that had been lost due to the neuronal lesion.

The presently claimed invention teaches the use of compounds that inhibit formation of basal lamina in order to treat the neuronal damage - and accompanying loss of CNS functioning - caused by lesions in the nervous system, e.g., the severing of a nerve.

Attached hereto (Appendix, pages i-v) is an experimental report showing the extraordinary recovery of nerve-damaged rats following treatment in accordance with the presently claimed invention. The fornix was damaged in one series of test rats, and in another series the spinal cord was damaged. In these experimental paradigms two different, but defined, fiber tracts were transected (fornix lesion: see drawings in WO 98/51708; spinal lesions, Appendix, Figs. 1 and 2). These experiments comprised both compartments of the central nervous system, i.e., the brain and the spinal cord, and focused on the axonal regeneration of two different cell types, (i.e. fornix: limbic system fiber tract, non-motor tract; spinal cord: corticospinal tract, motor tract) to show the universality of response (i.e., axonal regeneration) with respect to *cell type* in the treatment of CNS

lesions, as presently claimed, with basal-lamina-formation inhibitors (using prolyl 4-hydroxylase inhibitors as examples). In *each case* (fornix lesion: see drawings in WO 98/51708; spinal lesions, Appendix, Fig. 3) basal-lamina formation was *effectively inhibited* by local application of different iron chelators (fornix: 2,2'-bipyridine; spinal cord: 2,2'-bipyridine-5,5'-dicarboxylic acid and desferrioxamine) and anti-collagen type IV antibodies (fornix). The different agents used were, in fact, selected in order to determine the universality, with respect to neuronal-cell type, of effective treatment in accordance with invention presently claimed, i.e., by using prolyl 4-hydroxylase inhibitors to prevent basal lamina formation in damaged nerve tissue. For both brain-lesion and spinal-cord-lesion paradigms, administration of basal-lamina-formation inhibitors (i.e., prolyl 4-hydroxylase inhibitors) resulted in axonal regeneration (fornix lesion: see drawings in WO 98/51708; spinal lesions, Appendix, Figs. 4 and 5) and associated recovery of CNS functioning. The recovery of functioning by regeneration in the fornix lesion is shown, necessarily, by electrophysiological improvement (i.e., because there is no behavioral outcome measurable in fornix lesioned animals); whereas, the recovery of CNS functioning accompanying regeneration spinal lesion is demonstrated, directly, by locomotion testing (open field test, walking analysis performed by cat-walk testing) and by testing fine-motor movements based on sensory motor coupling (testing by walking on a horizontal ladder) (fornix lesion: see drawings in WO 98/51708; spinal lesions, Appendix, Figs.6-8).

Accordingly, the instant specification provides sufficient teaching to enable the skilled person to practice the invention as presently claimed, i.e., how to administer the substances that inhibit the

formation of the basal membrane and, so, effect neuronal regeneration with a corresponding recovery of CNS functioning.

The protocol described in the present specification for the brain-lesion model (i.e., the transected postcommissural fornix) is readily extrapolated to treatment of spinal-cord damage. This is demonstrated by behavioral testing (Appendix), which could be used because of the suitability of the lesion paradigm, wherein local application of, even, prolyl 4-hydroxylase inhibitors other than 2,2'-bipyridine (i.e., the inhibitors 2,2'-bipyridine-5,5'-dicarboxylic acid and desferrioxamine [deferoxamine]) leads to the same positive result (i.e., with regard to prevention of basal lamina formation and the promotion of axonal regeneration and functional recovery) as observed in connection with the brain-lesion model (discussed above).

With these two experimental setups the applicability of treatment in accordance with the presently claimed invention is shown for CNS lesions, in general. In each lesion paradigm, and for each inhibitor substance used, a dose-response test (which is the normal procedure for pharmacological testing) was necessary to determine the useful concentration of the inhibitor substance to be administered to prevent basal lamina formation. Therefore, applicants submit that the described protocol enables the skilled person to practice the presently claimed invention, e.g., reproducing the experiments performed and results obtained for the brain and spinal cord lesion paradigms.

Thus, the requirements of enablement under §112, ¶1, are satisfied in connection with the subject matter presently claimed.

The statement of rejection concludes that the instant specification does not teach a method or working example that indicates recovery of central-nervous-system functioning associated with the observed axonal regeneration. This conclusion reached is incorrect. It would have been readily understood by the skilled person that a successful axonal regeneration of damaged (lesioned) nerve tissue leads (as a natural incident) to the recovery of CNS functioning that had been lost due to the nerve damage suffered. Moreover, such functional recovery (as explained above) is demonstrated, (i) for the brain-lesion paradigm (transected postcommissural fornix), by the restoration of electro-physiological properties and, (ii) for the spinal-cord-lesion paradigm, by the recovery of locomotion skills (in cat-walk-testing) and fine-motor movements based on sensory motor coupling (in walking on a horizontal ladder).

Thus, no undue experimentation would have been required of the skilled artisan to determine useful inhibitors of basal cell membrane formation, in particularly, since classes of useful inhibitor compounds are expressly recited in the present claims. For example, amino acid hydroxylase is a well known enzyme, which plays a role in the formation of the basal lamina. Compounds in the class of inhibitors of amino acid hydroxylase are also well known, e.g., as disclosed in the present specification, such as bipyridyl Fe-ion chelators, among others. Such preferred compounds (embodiments) are expressly recited in present claims 49 and 56.

The statement of rejection further alleges that the present specification would not have enabled the skilled artisan to identify the cell types to which the presently claimed method is

applicable. The allegation is incorrect, as shown by the experimental report (Appendix), discussed above.

The presently claimed invention effects the recovery of functioning lost as the result of nervous system lesions. This recovery is the manifestation of axonal regeneration, i.e., promoted by inhibiting basal-lamina formation in accordance with the presently claimed invention, which occurs predominantly in *neuronal cells* (*Medline Plus, Medical Dictionary*, definition of "axon" "axon illustration," attached hereto as Exhibit A). Two, different neuronal cell types were tested, (i.e., fornix: limbic system fiber tract, non-motor tract; spinal cord: corticospinal tract, motor tract) to show the universality of cell-type response (i.e., axonal regeneration) to the treatment of CNS lesions with prolyl 4-hydroxylase inhibitors to prevent basal lamina formation. The universality is shown, especially since the corticospinal tract in the spinal cord responds to the treatment, because this fiber tract is regarded the most difficult regenerating fiber tract in the CNS in the field of regeneration research. For the brain and the spinal cord it was shown that regardless of which neuronal cell types are involved in the lesion, basal lamina formation occurs after injury and that there is significant axonal regeneration as well as behavioral improvement if basal lamina formation is prevented.

As disclosed in the present specification (¶ bridging pages 1 and 2), the presently claimed invention is effective for the treatment of lesions in the spinal cord, as well as lesions in the brain. This is, moreover, confirmed by the experimental report (Appendix) provided herewith, which

shows, in fact, the effective use of the presently claimed invention, in attaining neuronal regeneration and accompanying recovery of CNS functioning, in the treatment of spinal cord lesions.

With respect to dosage regimen, one skilled in the art would have known that the dosage has to be adjusted as low as possible in the treatment of brain cell damage, as well as knowing that, for spinal cord lesions, a higher dosage than used for brain-cell damage can be administered. It would have required only routine optimization to find out an optimum regimen for the specific treatments.

The allegation that enablement is satisfied only for *local* administration of the inhibitor substance is not well taken. The specification can satisfy the requirements for enablement under §112, 1, without containing a single working example. *In re Strahilevitz*, 212 USPQ 561 (CCPA 1982). Whether there are working examples for administering the systemically, orally, and intravenously is of no moment, since one skilled in the art would have readily known how to perform such administrations. The examples provided administer the inhibitor substance locally as a matter of convenience, i.e., because of practical obstacles to, e.g., systemic, oral, and intravenous administration.

That problems might exist with respect to certain embodiments that fall with the scope of the present claims, i.e., embodiments using proteins as the inhibitor substance, is insufficient grounds to reject the claims for alleged lack of enablement. For example, Deferoxamine is well known, approved drug for systemic administration in the treatment of thalassaemia, is a non-protein inhibitor of basal-lamina formation useful in the invention presently claimed.

Moreover, one skilled in the art would have known not to administer protein inhibitor substances orally but, rather, via the mucosa or by local administration. The instant specification provides sufficient enablement, not only to perform (repeat) the embodiments described therein, but also for the full scope of the present claims. Excluding from the claims embodiments known to be inoperative is not required by §112, ¶1. *In re Smythe*, 178 USPQ 279, 286 (CCPA 1973).

Favorable action is requested.

Respectfully submitted,

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axon
axon hillock
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Main Entry: **ax·on**
Pronunciation: 'ak-"sän
Variant(s): *also* **ax·one** /-"sōn/
Function: *noun*
: a usually long and single nerve-cell process that usually conducts impulses away from the cell body
- ax·o·nal /'ak-sən-əl; ak-'sän-, -'sōn-/ *adjective*
[axon illustration]

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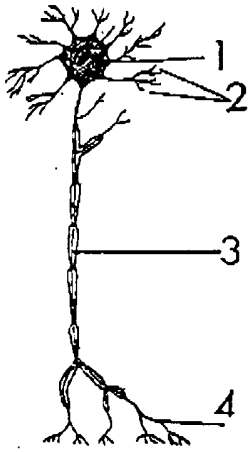

neuron: 1 cell body, 2 dendrite, 3 axon, 4 nerve ending

EXHIBIT A

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Main Entry: **ax·on**
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Function: *noun*
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- ax·o·nal /"ak-sən-ᵊl; ak-'sän-, -'sōn-/ *adjective*
[axon illustration]

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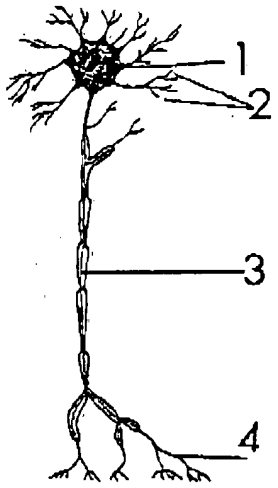

neuron: 1 cell body, 2 dendrite, 3 axon, 4 nerve ending

EXHIBIT A

Experiments in the Molecular Neurobiology Laboratory, Dept. of Neurology, Heinrich-Heine-University Duesseldorf, of Prof. Müller have shown that local application of iron chelators (different from 2,2'-bipyridine [syn.: α,α'-dipyridyl], namely a 5,5'-dicarboxylic acid derivative of 2,2'-bipyridine (BPY-DCA) and another iron chelator, namely deferoxamine) prevents basal lamina formation following spinal cord lesions thus enabling axonal regeneration and functional recovery of locomotor and fine motor movements in the adult rat.

In spinal lesions (Fig. 1, Fig. 2A) a basal lamina forms (Fig. 2A) as it has been shown in the deep brain lesion of the transected postcommissural fornix (Fig. 2B).

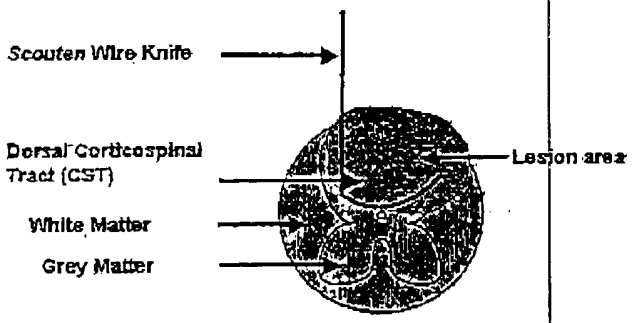

Fig. 1: Lesion of the dorsal part of the spinal cord with a scouten wire knife

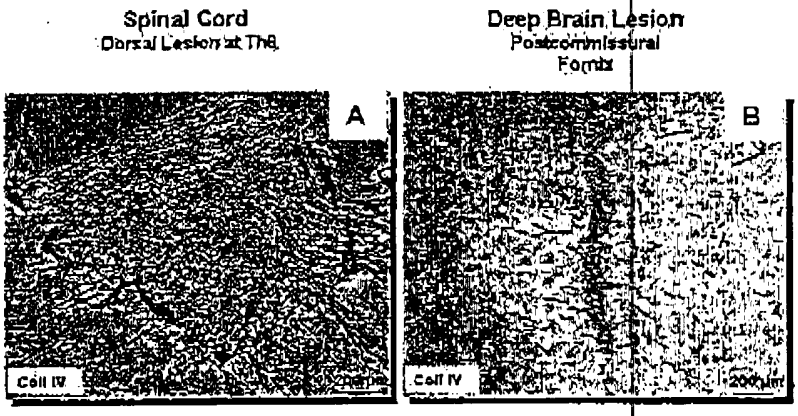

Fig. 2: Basal lamina formation visualized by immunohistochemical staining for Col. type IV in a dorsal spinal cord lesion (A) and the lesioned postcommissural fornix (B).

To show the universality of different prolyl 4-hydroxylase inhibitors we injected a 5,5'-dicarboxylic acid derivative of 2,2'-bipyridine (BPY/DCA) and another iron chelator, deferoxamine (syn: desferrioxamine, Fig. 3). The use of different prolyl 4-hydroxylase inhibitors states in our opinion the universal applicability of these substance classes. Dosage has to be established individually for each inhibitor.

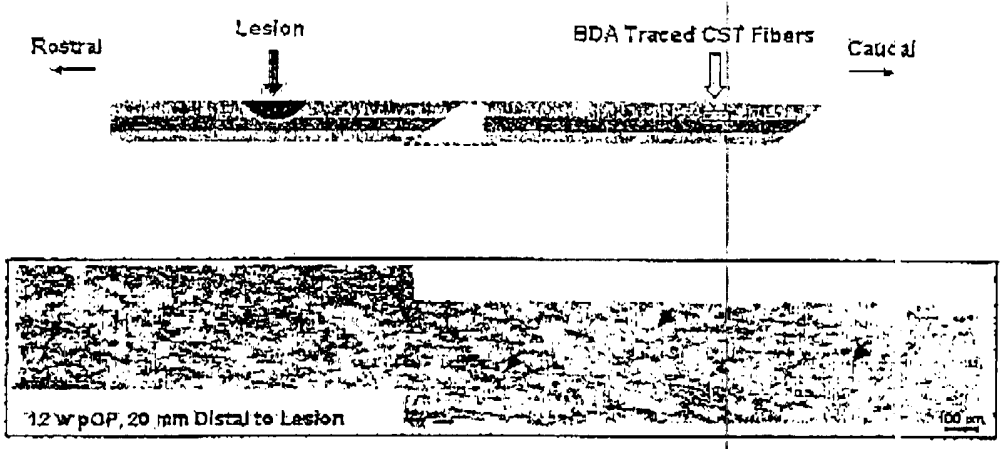

Fig. 5: Long distance regeneration in a parasagittal section taken from the lumbar spinal cord. BDA-traced CST fibers can be found 2 cm distal to the original lesion site.

Prevention of basal lamina formation by application of prolyl 4-hydroxylase inhibitors furthermore resulted in a significantly improved behavioural outcome of the tested animals. Animals were tested with regard to locomotion ability in the open field using the well established BBB-score, developed by Michelle Basso, Michael Beattie, and Jackie Bresnahan (all Ohio State University) (Basso et al., 1995). To evaluate fine motor movements, animals were tested on a horizontal ladder where footfalls were counted according to (Metz et al., 2000). Additionally the animals' walking pattern were evaluated using an automated quantitative gait analysis called "CatWalk"(Hamers et al., 2001).

In all three tested tasks the treated animals performed significantly better than control animals (Figs. 6-8).

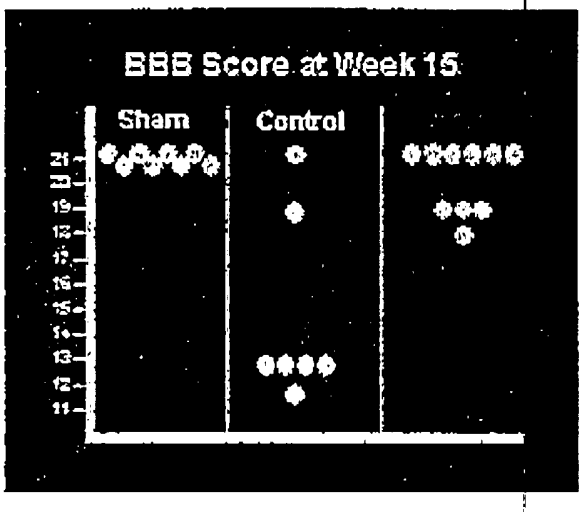

Fig. 6: BBB locomotor score 15 weeks post operation. Sham animals received the operation. A laminectomy was performed and the dura was opened but the spinal cord remained intact. Sham animals show the best possible locomotor performance receiving 21 points in the BBB-score. Control animals received buffer injections and show a general performance that does not allow the animals to perform coordinate walking pattern (BBB score 12 to 13). Animals

receiving the prolyl 4-hydroxylase inhibitor show a overall performance like sham operated animals 15 weeks post operation. All treated animals show coordinate walking pattern.

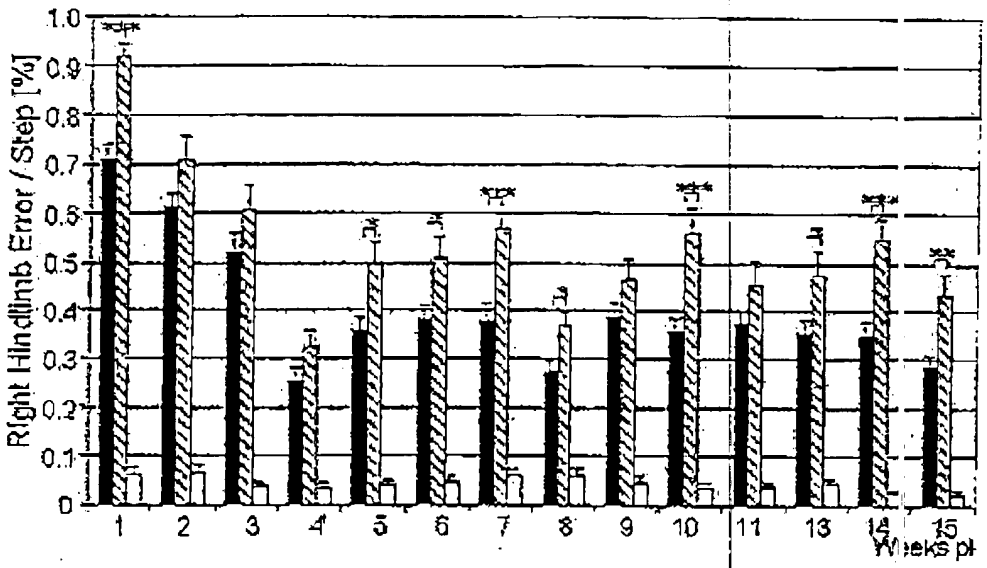

Fig. 7: Horizontal ladder test to evaluate fine motor movements. Filled (black) bars represent animals treated with prolyl 4-hydroxylase inhibitor, striped bars represent buffer control animals and blank bars represent sham operated animals. Treated animals perform significantly better (less footfalls) compared to controls.

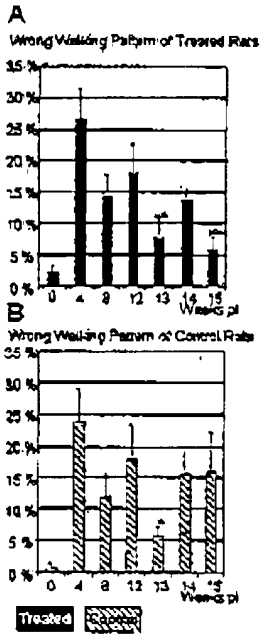

Fig. 8: (A) and (B) show results of the analysis of animals over ground locomotion on the CatWalk device. The mean percentage of wrong walking patterns +/- standard error of the mean before and after operation is shown for treated animals in (A) and for control animals in (B). Significant differences of data pl week 8 - 15 in comparison to pl week 4 are depicted by

APPENDIX

asterisks (* $p<0.05$, ** $p<0.005$, *** $p<0.001$). Treated animals show significant improvement of the regular walking pattern on several testing days, but control animals remain at a high level of irregular walking patterns, except for week 13. Additionally significant differences could be observed between pre-lesion baseline values and p week 15 and 16 for control animals ($p<0.05$), where treated animals show no significant difference between performance results of pre-lesion and pl week 15 and 16 performances, indicating a recovery to pre-lesion performance.

Reference List

1. Basso DM, Beattie MS, Bresnahan JC (1995) A sensitive and reliable locomotor rating scale for open field testing in rats. J Neurotrauma 12: 1-21.

2. Hamers FP, Lankhorst AJ, van Laar TJ, Veldhuis WB, Gispen WH (2001) Automated quantitative gait analysis during overground locomotion in the rat: its application to spinal cord contusion and transection injuries. J Neurotrauma 18: 187-201.

3. Metz GA, Merkler D, Dietz V, Schwab ME, Fouad K (2000) Efficient testing of motor function in spinal cord injured rats. Brain Res 883: 165-177.

APPENDIX

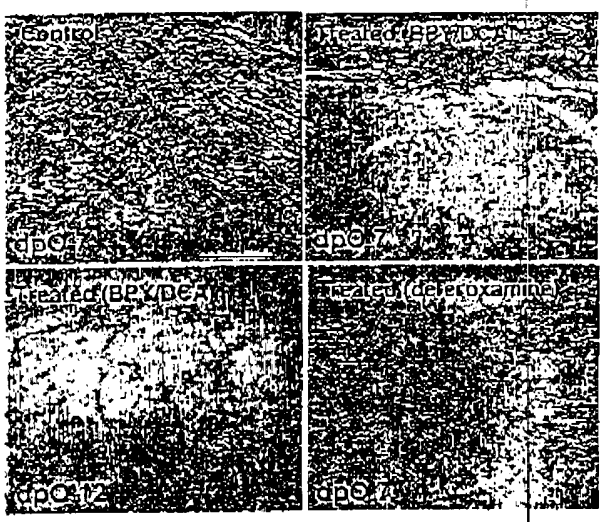

Fig. 3: Prevention of basal lamina formation in spinal cord lesions by immediate injection of different prolyl 4-hydroxylase inhibitors at 7 and 12 days post operation (dpo). Basal lamina formation visualized by immunohistochemical staining for Coll type IV. Magnification bar in control picture for all: 500 µm.

Prevention of basal lamina formation in the traumatically injured spinal cord resulted in axonal regeneration across the lesion site (Fig. 4) and long distance axonal elongation of regenerated fibers in the denervated distal spinal cord (Fig. 5). To visualize a specific fiber population (dorsal corticospinal tract, CST) a tracer substance (biotinylated dextran amine, BDA) was injected into the sensory motor cortex of the animals. This tracer substance is transported in fibers that have a connection to the cell bodies, i.e. if CST fibers in or beyond the lesion site carry the tracer these fibers are regenerated.

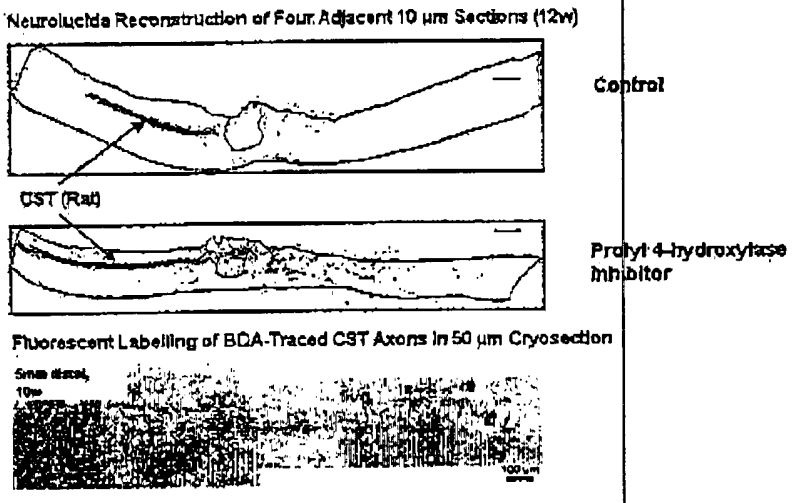

Fig. 4: Drawings: Neurolucida reconstructions of four adjacent parasagittal 10 µm sections, CST traced with BDA, 12 weeks post operation. In control animals transected, BDA-traced CST fibers do not enter the lesion area and do not elongate in the denervated distal part of the spinal cord. In animals treated with a prolyl 4-hydroxylase inhibitor transected, BDA-traced CST fibers cross the lesion site and elongate in the distal part of the spinal cord. Magnification bars: 1mm. Photomicrograph : Fluorescent labelling of regenerating BDA-traced CST fibers 5 mm distal to the lesion site, 10 weeks post operation.

APPENDIX